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RESEARCH PAPER

Effect of High Performance Inulin (Fructo oligosacharride) on Glycemic Control in Alloxan Induced Diabetic Rats

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ABSTRACT : The purpose of this study was to carried out to investigate the effect of inulin extracted from Jerusalem artichoke(Helianthus tuberosis)on decrease the blood glucose in alloxan induced diabetic rats. The oral administration of inulin decreased blood glucose , implying the utility of inulin as a bioactive material to prevent metabolic diseases oh humans. In the experiment, a total of 60 white rats were completely randomly allocated into 10 groups and six rats were used in each group (54 diabetic rats and six normal rats) were used. Diabetes was induced three days before starting the experiment. Concerning growth performance, in comparison with the control group, daily weight gain in the inulin administrated rats increased and recovered to the normal level. The average weight gain in treated group ranged from 19 g (T_1) to 36g (T_2I) after 28 days of study period. Blood glucose was significantly lowered in the inulin administrated group from 204.16 ± 3.57 to 121.16 ± 3.4 mg/dL (a reduction of 40.65%) at the end of four weeks feeding trials.

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Inulin, a non-digestible carbohydrate, is a fructan that is not only found in many plants as a storage carbohydrate, but has also been part of man's daily diet for several centuries. It consists of a long chain made up of 22-60 fructose molecules linked to each other by β (2→1) bonds, with a terminal glucose molecule. It is found in a variety of plant sources such as Jerusalem artichoke, chicory, dahlias, onion, garlic, banana, asparagus and leek. Amongst these vegetables, chicory roots are outlined for inulin production in industrial scale due to its higher content and stability in producing high Fructose/ Glucose ratio andbecause of its regular growing, even in moderate climates (Abrams *et al.*, 1982 and Bajpai and Bajpai, 1991).

Inulin is mostly commercialized as a powder, which provides for convenient manipulation, transportation, storage and consumption. Degree of polymerization ranges from 2 to 60 depending on the source of inulin, time of harvest and the process of production (Champman and Champan, 1980). Inulin can act as a sugar or fat substitute, with the advantage of exhibiting a very low caloric value. It used as an ingredient in foods with reduced or no sugar and fat, such as chocolates, ice creams and

yogurt. Pure inulin is slightly sweet (10% sweetness in comparison with sugar), whereas high performance inulin (with a degree of polymerisation greater than ten) is tasteless. It acts in the human organs in a similar way as dietary fibres, contributing to the improvement of the gastrointestinal system conditions (Jerums *et al.*, 2003). Many food and pharmaceutical industries have found applications for inulin in the production of functional foods, nutritional composites and medicines.

Inulin is resistant to digestion in the upper gastrointestinal tract and it reaches the large intestine essentially intact, where it is fermented by colonic bacteria causing an increase in fecal biomass, production of short-chain fatty acids and decrease in cecocolonic pH. Hence, it is classified as a soluble dietary fibre (Murphy, 2001).

Diabetes mellitus(DM) is a metabolic disease that has glucose excreted with urine and causes disorders of protein, lipid and electrolyte metabolism as glucose in the blood increases, resulted from disorders of carbohydrate metabolism in the body due to short of insulin secreted from β- cells of langergans inlets in the pancreas (Abrams *et al.*,1982 and Wolfl *et al.*, 2005). DM is classified as type 1 DM which is insulin dependent and which is non- insulin dependent injections and type 2 DM which is non-insulin dependent an diets caused by increased insulin resistance in the affected peripheral tissues of muscles, liver and fat cells by living habits such as obesity, lack of exercise, smoking and alcohol consumption. DM typically causes disorders of lipid metabolism such increased Triacylglycerides (TAG) in the blood, total cholesterol, low density Lipoprotein Cholesterol (LDLC) and atherogenic index and decreasing High Density Lipoprotein Cholesterol (HDLC) which result in serious complications (Steinberg *et al.*, 1996 and Treadway *et al.*, 2001). Dm may increase death rate due to complications such as cerebrovascular or cardiovascular diseases or hypertension caused by decreased activities of anti- oxidative enzymes and resulting increased oxidative stresses in the body (Champman and Champman, 1980 and Jerums *et al.*, 2003).

In recent times, fortified foods with purified dietary fibre or the direct addition of fibre rich source to improve the nutritional quality of food product are encouraged owing to health benefits associated with dietary fibre. Purified dietary fibres are available in the form of pure cellulose, hemicellulose, xylan, raffinose, pectin, guar gum, sodium alginate, carrageenan, carob bean gum, psyllium husk, inulin, resistant starch or their mixtures. Similarly, fibre rich sources are wheat bran, oat bran, sorghum meal, barley hulls, barley husk, soyabean hulls, pea hulls, canola hulls, fruits and vegetables (Nayak *et al.*, 2010).

The plant whose contents are 14-19 per cent inulin has emerged newly as a beneficial constituest if anti-diabetic prebiotics whose bioactivities include reduction of blood lipids, prevention of intestinal diseases, improvement of constipation and hypoglycemic activity

Table A : Effect of Spirulina supplementary on Triglyceride of HIV infected patient

Gender	Treatments	Parameter	Mean before	Mean after	Mean difference	S.E. of difference	teal	'p' value
Male	T_0	Triglyceride	154.95	176.3	+21.35	8.158	2.62	0.0127 *
	T_1	Triglyceride	160.9	149.39	-11.51	8.965	2.288	0.027 *
Female	T_0	Triglyceride	163. c	180.11	+ 17.11	14.16	1.21	0.245 IMS
	T_1	Triglyceride	167.4	150.7	-16.7	10.49	1.994	0.047 *

* indicate significance of value at P=0.05, NS = Non-significant, The result is significant at $p < 0.05$, T_0- Control, T_1- Spirulina supplementation

Table B : Effect of Spirulina supplementary on Serum cholesterol level of HIV infected patient

Gender	Treatments	Parameter	Mean before	Mean after	Mean difference	S.E. of difference	teal	'p' value
Male	T_0	Serum cholesterol	175	187.6	+12.6	9.112	1.4102	0.166 NS
	T_1	Serum cholesterol	179.5	164.7	-14.8	9.426	1.57	0.124 NS
Female	T_0	Serum cholesterol	182.78	191.44	+8.66	12.17	0.712	0.487 NS
	T_1	Serum cholesterol	190.5	177.5	-13	11.97	1.086	0.29 NS

Significant at P=0.05 level, NS = Non-significant, The result is significant at $p < 0.05$, T_0- Control, T_1- Spirulina Supplementation

(Bajpai and Bajpai, 1991 and Fiordaliso *et al*., 1995).

Inulin is officially recognized as a natural food ingredient in all European Union and has a self-affirmed Generally Recognized as Safe (GRAS) status in United States (Tungland, 2000). Inulin has a neutral taste, is colorless, and has minimal influence on the sensory characteristics of a product. Since addition of inulin does not contribute to any viscosity, it can be regarded as an invisible way of incorporating fibre to foods. It is an ingredient of the fructans that meet the needs of the food industry today, and are on the leading edge of the emerging trend towards functional foods (Frank, 2002). Hence, the objectives of this work was analyzed the effect of inulin of blood glucose control through animal study.

RESEARCH METHODOLOGY

Inulin :

Frutafit HD inulin was purchased from DKSH India private limited, Mumbai and it was stored in airtight container in refrigerated condition till used.

Wistar albino rats :

Wistar albino rats weighing 180-220 g were obtained from Thiruvananthapuram Medical College, Thiruvananthapuram, India and the study was conducted in K.M. College of Pharmacy, Madurai. The experiments were approved by the institutional animal ethics committee .The wistar albino rats were housed in large spacious cages and they were fed with multigrain mixes puffed snack food and access to water *ad libitum*. The animals were acclimatized to the standard environmental condition of temperature (22°C±5°C) and humidity (55±5%) and 12 hrs light and dark cycles throughout the experimental period.

Preparation of animal :

Diabetes mellitus was induced in wistar albino rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150 mg/kg BW) in physiological saline after overnight fasting for 12 hrs.

Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2, O_2 and HO^-. The development of hyperglycemia in rats is confirmed by plasma glucose estimation on 72 h post alloxan injection. The rats with fasting plasma glucose level of >150 mg/dL were used for this experiment.

Animal experimental protocol :

In the experiment, a total of 60 rats (54 diabetic rats and six normal rats) were used. Diabetes was induced three days before starting the experiment. The rats were divided into 10 groups (n=10 rats/ group) after inducing diabetes. In the experiment, six rats were used in each group.

Treatment protocol :

– Group-I: (Control) consist of normal rats.

– Group-II: Control (diabetic control) given 150 mg/kg of BW, Alloxan monohydrate through intraperitoneal to induce diabetes.

Treatment groups:

Experiments I to X were run for 28 days

– Group-III: Diabetic rat given only multigrain mix extruded snack food (T_1).
– Group-IV: Diabetic rat given only multigrain mix with inulin extruded snack food (T_5I).
– Group-V: Diabetic rat given only multigrain mix extruded snack food (T_2).
– Group-VI: Diabetic rat given only multigrain mix with inulin extruded snack food (T_6I).
– Group-VII: Diabetic rat given only multigrain mix extruded snack food (T_3).
– Group-VIII: Diabetic rat given only multigrain mix with inulin extruded snack food (T_7I).
– Group-IX: Diabetic rat given only multigrain mix extruded snack food (T_4).
– Group-X: Diabetic rat given only multigrain mix with inulin extruded snack food (T_8I).

Sample collection :

At the start and after 28 days of feeding trial, the blood glucose level and body weight were measured. Then blood was collected retro-orbitally from the eye under light ether anesthesia using capillary tubes. Blood was collected in fresh vials containing EDTA as anticoagulant agent and serum was separated in a T_8 electric centrifuge at 2000 rpm for two minutes. Then serum samples were used for various biochemical tests (Al- shamaony *et al*., 1994).

RESULTS AND DISCUSSION

The effect of inulin on the body weight of the control

and experimental animals. The initial weight of the various groups of rats ranged from 189.86±4.51 to 211.66±5.72 g. After 28 days of study there was a statistically significant increase in the body weight of the rat in normal (G1) and treatment groups (G3-G10) while the toxic control (G2 which were the rats with diabetes but not given any treatment) showed decrease in weight. The average weight gain in treated group ranged from 19 g (T_1) to 36g (T_2I) after 28 days of study period. The multigrain mixes brought about weight gain to different extent in all the treatment groups.

During digestion, wave like currents caused by contractions of the intestinal muscles bring nutrients to the surface of the intestinal wall for absorption. After soluble fibre dissolves in water, however, it traps nutrients inside its gummy gel and slows down considerably while moving through the digestive tract. Inside the gel, nutrients are shielded from digestive enzymes and less likely to reach the wall of the intestines. Dietary sugars like carbohydrates and starch are among the nutrients trapped inside this gel. Consequently, sugar is absorbed into the bloodstream more slowly, blunting the sharp hike in blood glucose typically experienced by diabetic patients after a meal. Lesser hike in blood glucose lead to greater

Table 1 : Effect of MGM product (puffed snack) on body weight

Groups	Initial body weight (g)	Final body weight (g)	Changes in body weight	
			Gram	Percentage
Group I (G 1)	202 ± 4.09	206.33 ± 3.10	4.00	1.98
Group II (G 2)	195.83 ± 4.72	157.33 ± 2.41*[a]	-39	-19.91
Group III (G 3)	202.5 ± 3.81	221.33 ± 5.54*[b]	19	9.38
Group IV (G 4)	206.66 ± 5.72	227.33 ± 3.86*[b]	21	10.16
Group V (G 5)	196.66 ± 4.41	221.0 ± 1.80*[b]	24.34	12.37
Group VI (G 6)	211.63 ± 4.52	247.23 ± 3.56*[b]	36	17.01
Group VII (G 7)	189.86 ± 4.51	214.0 ± 1.90*[b]	24.14	12.71
Group VIII (G 8)	211.66 ± 5.72	233.33 ± 3.46*[b]	22	10.39
Group IX (G 9)	210.66 ± 5.42	237.33 ± 3.76*[b]	27	12.81
Group X(G 10)	192.66 ± 4.31	227.0 ± 3.70*[b]	34.34	17.82

G1- Normal control, G2- Diabetic control, G3- Treatment group (T_1), G4- Treatment group (T_5I), G5- Treatment group (T_2) ; G6- Treatment group (T_6 I), G7- Treatment group (T_3), G8- Treatment group (T_7 I), G9- Treatment group (T_4), G10- Treatment group (T_8I).
*a values were significantly different from normal control (G 1) at (P<0.01)
*b values were significantly different from diabetic control (G 2) at (P<0.01)

Table 2 : Effect of MGM product (puffed snack) on glucose levels (mg/dl) in alloxan induced diabetic rats

Groups	0^{th} Day	14^{th} Day	28^{th} Day	Changes in glucose levels *	
				Gram	Percentage
Group I (G 1)	71.5 ± 5.67	70.83 ± 5.38	66.33 ± 4.42	5.17	7.26
Group II (G 2)	148.66 ± 5.48	166.16 ± 6.18*[a]	213.5 ± 7.43*[a]	64.84	43.61**
Group III (G 3)	183.5 ± 4.74	145.33 ± 4.99*[b]	129.66 ± 3.20*[b]	53.84	29.34
Group IV (G 4)	193.16 ± 3.67	153.33 ± 4.47*[b]	117.16 ± 3.41*[b]	76.00	39.34
Group V (G 5)	189.83 ± 4.57	157.16 ± 4.40*[b]	132.16 ± 3.14*[b]	57.67	30.37
Group VI (G 6)	204.16 ± 3.57	150.33 ± 3.47*[b]	121.16 ± 3.47*[b]	83.00	40.65
Group VII (G 7)	197.83 ± 4.37	159.16 ± 4.50*[b]	138.16 ± 3.34*[b]	59.67	30.16
Group VIII (G 8)	198.16 ± 3.47	142.33 ± 3.37*[b]	118.16 ± 3.66*[b]	80.00	40.37
Group IX (G 9)	185.83 ± 4.16	151.16 ± 4.26*[b]	138.16 ± 3.43*[b]	47.67	25.65
Group X(G 10)	195.83 ± 4.37	146.16 ± 3.38*[b]	120.16 ± 3.23[b]	75.67	38.64

* The difference of 0^{th} day and 28^{th} day values were taken for percentage calculation.
** The value indicates the percentage increased in glucose level.
G1- Normal Control, G2- Diabetic Control, G3- Treatment group (T_1), G4- Treatment group (T_5I), G5- Treatment group (T_2), G6- Treatment group (T_6 I), G7- Treatment group (T_3), G8- Treatment group (T_7 I), G9- Treatment group (T_4), G10- Treatment group (T_8I).
*a values were significantly different from normal control (G 1) at (P<0.01)
*b values were significantly different from diabetic control (G 2) at (P<0.01)

sensitivity to the action of insulin. Avoiding high peaks and low valleys in blood glucose places less stress on the pancreas and is important not only to diabetics but also to those who want to prevent the development of type 2 diabetes.

Scientists propose one other explanation for soluble fibre's effect on blood glucose. In order for nutrients to be absorbed into the intestines, they must first cross an unstirred water layer covering the surface of the intestines. Soluble fibre thickens this layer, making it more resistant to the movement of nutrients diffusing into the body. Both theories attempt to explain why blood glucose levels rise more slowly when consumed with soluble fibre (Chandalia *et al*., 2000).

The effect of four week feeding on the glucose levels (mg/dL blood) in diabetic rats. The initial blood glucose level of the normal (G1) and toxic control (G2) rats was 71.5±5.67 and 148.66±5.48 mg/dL blood respectively. There was a statistically significant reduction in the blood glucose at the end of four weeks feeding trials. The maximum reduction in blood glucose was found in G6 rats, fed with T_6I sample where initial glucose levels reduced from 204.16 ± 3.57 to 121.16 ± 3.4 mg/dL (a reduction of 40.65%). Rumessen *et al.* (1990) observed a lowering of blood glycemic response and peak insulin levels when 10g of artichoke inulin was added to 50g of wheat-starch meal of healthy human subjects.

The mechanism mediating the hypocholesterolemic properties of soluble fibre, firstly may displace other cholesterol raising dietary components such as saturated fatty acids and by contributing to satiety, may influence the quantity or timing of food intake (Jenkins *et al*.,1989 and Swain *et al*.,1990). Secondly, some fibres may bind bile acids and interfere with micelle formulation in the small intestine. This could alter the quantity or location of fat and cholesterol absorption and possibly lead to an increase in bile acid excretion (Jenkins *et al*., 1993). Thirdly, short chain fatty acids produced by bacterial fermentation and absorbed into the portal blood supply may have an inhibitory effect on hepatic cholesterol synthesis (Chen *et al*., 1984).

Measurement of atherogenic index (AI), is a measure of the atherogenic property of an agent, was calculated using the following formula, AI = (Total cholesterol – HDL-C)/HDL-C. Atherogenic property refers to the ability to initiate or accelerate atherogenesis which is the deposition of atheromas, lipids and calcium in the arterial lumen (McGowan *et al*., 1983)

Measurement of glycosylated haemoglobin (HbA1C) reflects a weighted average of plasma glucose concentration over the preceding weeks, thereby complementing day-to-day testing. In nondiabetic persons HbA1C values are four per cent to six per cent; these values correspond to mean blood glucose levels of about 90 mg/dL (or about 5 mmol/L). An HbA1C of six per cent reflects an average plasma glucose level of ≈ 120 mg/dL. In general, each one per cent increase in HbA1C is a reflection of an increase in average glucose levels of ≈30 mg/dl (Franz, 2008)

The plasma insulin, haemoglobin and glycosylated haemoglobin (HbA1C) were assessed after the feeding trial. The plasma insulin level in normal control rats which was 25.21±0.54 µu/ml. Plasma insulin level of G4, G6 and G8 were close to that of G1. The G2 rats which were diabetic induced, and with no treatment given, had the least plasma insulin of 11.83±0.19 µu /ml. Except G2 group the plasma insulin values for all the groups were found to be higher indicating that the treatments had a positive effect on insulin levels. Glycosylated Hb level of the treatment groups in (G3-G10) was lower than the diabetic control group, though more than that of normal control rats. Rats given inulin containing MGM snack food had lower HbA1C indicating a hypoglycemic effect due to the inclusion of inulin. Presence of kodo millet, horse gram and bengal gram appeared to have an appreciable hypoglycemic effect on the diabetic rats.

The results proved that the inulin incorporated multigrain mixes exerted a better hypoglycemic effect when compared to the multigrain mixes without inulin. Of the inulin included multigrain mixes T_6I samples exhibited the highest effect which was evident from the increase in body weight, reduction in blood glucose level, total and glycosylated Hb levels of the animals.

Today's consumers hold high standards for the foods they consume. They demand foods that taste great, fat and calorie reduced, and they are interested in foods that provide added health benefits. It is also expected that these foods will be convenient and affordable. The desire of consumers to look good and stay healthy in a fast-paced environment is becoming more difficult to fulfill. Quick fixes and shortcuts are attractive to the consumer, whether they refer to food preparation, weight loss or disease prevention. Consumers are also more informed and more aware of the links between diet and health than ever before. Consequently they are looking for foods to provide multiple benefits as well as good taste. This

speaks of a strong focus on disease prevention and indicates that the time is right for optimizing health. By and large functional foods can be defined as the food that beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either the state of well being and health or the reduction of the risk of a disease and inulin can be regarded as functional food.

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